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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US97/04627 (22) International Filing Date: 21 March 1997 (21.03.97) (30) Priority Data: 08/622,516 25 March 1996 (25.03.96) US (60) Parent Application or Grant (63) Related by Continuation US 08/622,516 (CIP) Filed on 25 March 1996 (25.03.96) (71) Applicant (for all designated States except US): SANGSTAT MEDICAL CORPORATION [US/US]; 1505-B Adams Drive, Menlo Park, CA 94025 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): FLOC'H, Robert [FR/FR]; 9, avenue d'Auray, F-44300 Nantes (FR). MERLE, Christian [FR/FR]; 7, impasse Sainte-Radégonde, F-86000 Poitiers (FR). (74) Agents: TRECARTIN, Richard, F. et al.; Flehr, Hohbach, Test, Albritton & Herbert L.L.P., Suite 3400, 4 Embarcadero Center, San Francisco, CA 94111-4187 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: CYCLOSPORIN A FORMULATIONS AS NANOPARTICLES		
(57) Abstract Cyclosporin A formulations are provided as amorphous nanoparticle dispersions for physiologic absorption. The compositions have high bioavailability and patient acceptability. By providing for concentrates comprising lower alkanols and a polyoxyalkylene surfactant as a stable dispersion of cyclosporin A, upon introducing the stable dispersion into an aqueous medium, the subject formulation is produced comprising amorphous bioavailable cyclosporin nanoparticles.		

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CYCLOSPORIN A FORMULATIONS AS NANOPARTICLES

BACKGROUND OF THE INVENTION

The drug cyclosporin A, despite its many shortcomings and the difficulties in formulation, variations in bioavailability, and side effects, has proven to be one of the great success stories of the drug industry. Because of cyclosporin A's

5 hydrophobicity, formulations of cyclosporin A must take into account the need for a stable dispersion of the cyclosporin A, as well as the manner of administration of the formulation. For example, if it is intended that the formulation be diluted with water prior to its being taken orally, the resulting composition must provide the cyclosporin A in a bioavailable form, where adverse effects are not enhanced,

10 preferably diminished. The cyclosporin A which will come out of solution should be dispersable, so that the dosage is repeatable. Alternatively, where the formulation is provided in a manner where the cyclosporin A formulation becomes diluted with gastric juices, such as the use of capsules, it is essential that the cyclosporin A retains its bioavailability and activity in the environment of the

15 gastric juices. In all events, the cyclosporin A must be able to be transported into the vascular system, where it can diminish the immune response.

It is therefore of interest to develop formulations which are organoleptically acceptable, provide for desirable levels of bioavailability, do not introduce adverse effects associated with cyclosporin A, and generally fulfill the requirements of

20 therapeutic formulations.

BRIEF SUMMARY OF THE INVENTION

Aqueous dispersions of cyclosporin are provided by introducing a stable dispersion of cyclosporin in a formulation comprising as co-solvents a lower alkanol and a polyoxyalkylene surfactant, and desirably a polyethylene glycol, as
5 co-solvent. Upon dilution of the stable dispersion, an aqueous dispersion is obtained comprising nanoparticles of cyclosporin in amorphous form having good bioavailability.

DETAILED DESCRIPTION

10 Methods and compositions are provided for producing an aqueous colloidal dispersion of cyclosporin nanoparticles having good bioavailability. The nanoparticles are substantially spheric, the cyclosporin is present in an amorphous form, and the average size will generally be less than about 1000 nm, greater than about 50 nm, generally in the range of about 200 - 800 nm, usually in the range of
15 about 200-600 nm. Generally, at least about 50 weight percent of the total weight of cyclosporin will be present as particles in the indicated size range. Larger particles may be present, particularly as aggregates of nanoparticles, where the average diameter will usually be less than about 50 μ m, more usually less than about 25 μ m, the aggregates usually not exceeding 40 weight % of the total
20 cyclosporin..

The amount of cyclosporin A amorphous particles in the composition will be sufficient for therapeutic effect. Since the formulation may be formed by introduction into an aqueous medium prior to administration or directly into the gastric juices, the particular concentration cannot be stated, since the dilution in the
25 stomach is uncertain. For preparation in an aqueous medium prior to oral administration, generally, the cyclosporin will be present at a weight percent of about 0.01-2.5, more usually from about 0.01-0.5 weight percent. The temperature of mixing may be in the range of about 10 to 50°C, usually in the range of about 20 to 40°C. Usually the mixing will involve stirring for sufficient
30 time to provide the solution of the cyclosporin.

The colloidal amorphous suspension of the nanoparticles is sufficiently stable to allow for some standing prior to administration, frequently up to about 6 hours, more frequently up to about 3 hours.

While cyclosporin A finds primary use, any of the cyclosporins which are physiologically acceptable, e.g. A through Z, may be employed.

The amorphous cyclosporin colloidal dispersion may be produced by preparing a stable dispersion of cyclosporin in a lower alkanol and a
5 polyoxyalkylene compound, either ester or alcohol. The alkanols will be ethanol or propylene glycol, individually or in combination, particularly where ethanol will be present in the range of about 25-75 volume percent, when the combination of alkanols is employed. The particular manner in which the colloidal dispersion is produced is not critical, so long as the materials used in the stable dispersion are
10 physiologically acceptable, do not interfere with the activity of the cyclosporin, and are readily available.

Various polyalkyleneoxy compounds may be employed which may serve as surfactants and co-solvents with the lower alkanols. The polyalkyleneoxy compounds are, therefore, liquids, soluble in both water and lower alkanols, have
15 low toxicity and in conjunction with the lower alkanols are capable of maintaining a stable dispersion, usually a solution of cyclosporin A. Exemplary of polyoxyethylene surfactants are polyoxyethylene esters, such as polyoxyethylene substituted sorbitan esterified with a fatty acid of from 12-18 carbon atoms, more usually from about 16-18 carbon atoms, exemplified by polysorbate 80. The
20 number of oxyethylene groups will generally be from about 10-30. Exemplary of polyoxyalkylene compounds as cosolvents are polyethylene glycols of an average molecular weight of less than about 2000, preferably less than about 1000, at least about 300, more usually in the range of about 300-700 particularly from about 350-500 kiloDaltons. Generally, greater than 50% by weight of the
25 polyethylene glycol will be within 50% of the average molecular weight of the polyethylene glycol.

In the formulation, the total amount of lower alkanol will generally be in the range of about 25-60 weight percent, more usually in the range of about 30-50 weight percent. The total amount of alkyleneoxy compound(s) will generally be in
30 the range of about 20-50 weight percent, more usually in the range of about 25-40 weight percent. Where combinations of polyoxyalkylene compounds are employed, the amount of the fatty acid ester will generally range from about 25-100% of the polyoxyalkylene compounds.

The weight of cyclosporin in the formulation will be sufficient to provide for a therapeutic dosage, generally in the range of about 2.5 to 25 weight percent, more usually in the range of about 5-15 weight percent.

The subject compositions may be prepared by first dissolving the cyclosporin in the lower alkanol, where a small proportion of the polyoxyalkylene compound may also be included, generally less than about 50 weight percent of the composition used for dissolving the cyclosporin. An elevated temperature may be employed, usually in the range of about 60 to 90°C. After dissolving the cyclosporin, the major proportion of the polyalkyleneoxy compound may be added and the total formulation brought to the desired ratios by the addition of the appropriate components. Generally, the cyclosporin can be dissolved in the lower alkanol (optionally including a portion of the polyalkyleneoxy compound) at a weight ratio of about 1:1.5-5, more usually 1:2-4.

The subject formulations may be used in accordance with conventional ways already described in the literature. Oral formulations have been reported in U.S. Patent Nos. 4,388,307; and 5,342,625; and UK Patent No. 2,222,770B, whose disclosures are incorporated herein by reference as describing the use of cyclosporin in oral formulations. Thus, the subject compositions may be administered as liquid solutions, capsules, or the like, taken orally in single or multiple dosages, as therapeutically required in accordance with conventional procedures. The formulations are used with patients who require that they be immunocompromised, as in the case of transplantation, autoimmune diseases and the like.

For convenience of the user, kits may be provided having the appropriate amount of cyclosporin, one or more dosage levels and the cosolvents, namely the lower alkanol(s) and the polyalkyleneoxy compound(s), e.g. at least one of ethanol and propylene glycol and at least one of polysorbate 80 and PEG400.

The following examples are offered by way of illustration and not by way of limitation.

Example 1

5 g of cyclosporin A was added to 5 mL of ethanol. The mixture was stirred to complete dissolution of cyclosporin A. To the resulting solution were

added 25 g of polysorbate 80 and the volume is completed to 50 mL by 1,2-propylene glycol. The mixture was sufficiently stirred at room temperature until a homogeneous solution was formed.

5 Example 2

5 g of cyclosporin A was added to 5 mL of ethanol. The mixture was stirred until complete dissolution of cyclosporin A. To the resulting solution were added 15 g of polysorbate 80 and the volume is completed to 50 mL by a mixture of 1,2-propylene glycol and polyethylene glycol 400. The mixture was sufficiently
10 stirred at room temperature until a homogeneous solution was formed.

Example 3

1 mL of the solution obtained in example 1 was added in 50 mL of water with a glass syringe as recommended for the oral administration of concentrated
15 emulsions or microemulsions in human. The addition of the solution was followed by a quick dissolution and a white suspension of fine particles was obtained having a blue reflect as colloidal suspensions (Tyndall effect). After centrifugation at 26,000 g during 5 hours, the sediment was washed with water and then centrifuged at 26,000 g during 24 hours. The washing and centrifugation processes were
20 repeated twice under the same conditions. After drying, an x-ray powder diagram was performed. The solid was exclusively in amorphous form.

The sediment was examined by scanning electron microscopy. The sediment was constituted of amorphous spheric nanoparticles with a diameter between 200 and 400 nm with the presence of some aggregates.

25 2 mL of the solution obtained in example 1 was added in 100 mL of water and the colloidal suspension was examined 10 minutes and 1 hour after the dilution by a diffraction/diffusion laser granulometer (Malvern SB.OD).

After 1 hour, two particle populations were observed: one representing 70% of the weight of cyclosporin A with an average diameter of 300 nm and a second
30 one representing 30% of the weight of cyclosporin A with an average diameter of 20 μ m, probably constituting aggregates of nanoparticles.

Example 4

1 mL of the solution obtained in example 1 was added to 50 mL of water and the colloidal suspension was stirred during 10 minutes.

The suspension was then added to 200 mL of artificial acidic gastric juice and warmed at 37°C. The homogeneous colloidal suspension was examined by diffraction/diffusion laser granulometry (Malvern SB.OD). The suspension was constituted exclusively of nanoparticles with an average diameter of 600 nm.

Example 5

10 1 mL of the solution obtained in example 1 was added directly to 200 mL of artificial acidic gastric juice.

The homogeneous suspension was warmed at 37°C and examined rapidly by diffraction/diffusion laser granulometry (Malvern SB.OD). The suspension was exclusively constituted of nanoparticles with an average diameter of 350 nm.

15

The subject cyclosporin compositions provide for excellent bioavailability in being amorphous particles, small, so as to have high surface area, and without detrimental effects other than those conventionally found with cyclosporin.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto

WHAT IS CLAIMED IS:

1. An aqueous dispersion of cyclosporin nanoparticles, wherein at least 50 weight percent of the cyclosporin present in the dispersion is of particles less
5 than about 1 μm , said cyclosporin being amorphous.
2. A dispersion according to Claim 1, comprising in minor amounts lower alkanol and at least one polyoxyethylene surfactant.
- 10 3. A dispersion according to Claim 2, wherein said polyoxyethylene surfactant is polysorbate 80.
4. A dispersion according to Claim 2, wherein said lower alkanol is at least one of ethanol and propylene glycol.
15
5. A dispersion according to Claim 1, comprising a polyethylene glycol of less than about 2000 Daltons.
- 20 6. In a method for orally administering cyclosporin to a patient, the improvement which comprises:
providing said cyclosporin, wherein at least 50 weight % of said cyclosporin is as amorphous particles of less than about 1000nm.
- 25 7. A method according to Claim 6, wherein said providing comprises adding to an aqueous medium a composition comprising cyclosporin dispersed in a combination of lower alkanol consisting of at least one of ethanol and propylene glycol and a polyoxyethylene surfactant.
- 30 8. A method according to Claim 7, wherein said polyoxyethylene surfactant is polysorbate 80.
9. A method according to Claim 7, wherein said lower alkanol is present in from about 25 to 60 weight percent, said polyoxyalkylene surfactant is

present in from about 20 to 50 weight percent, and said cyclosporin is present in from about 2.5 to 25 weight percent.

10. A method according to Claim 7, wherein said composition further
5 comprises polyoxyethylene cosolvent of less than 2000 Daltons.

11. A kit comprising cyclosporin, at least one of ethanol and propylene glycol, and polysorbate 80.

10 12. A kit according to Claim 11, further comprising PEG400.

13. A method for preparing a formulation according to Claim 1 comprising:

combining at least one of ethanol and propylene glycol with cyclosporin A
15 to from a solution; and

combining said solution with a polyethyleneoxy surfactant to form a second solution, which upon dilution with water forms amorphous nanoparticles of said cyclosporin A.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/04627

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 38/13

US CL : 424/450; 514/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/450; 514/9, 11, 938; 530/317, 321

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,996,193 A (HEWITT et al) 26 February 1991 (26/02/91), see column 4, lines 16-23, column 15, lines 1-4.	11, 13
X	US 5,047,396 A (ORBAN et al) 10 September 1991 (10/09/91), see column 2, lines 27-35 and 66-68, column 3, lines 1-10 and 52-59, column 4, line 46.	1, 2, 4 - 7, 9, 10, 13
X	US 5,154,930 A (POPESCU et al) 13 October 1992 (13/10/92), see column 9, lines 44-58, column 12, lines 18-31, Example 3.	1, 5, 6
X	US 5,206,219 A (A. DESAI) 27 April 1993 (27/04/93), see column 2, lines 15-16, 23-29, 51-53, column 3, line 56 - column 4, line 20, examples 1-5.	11, 12

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,474,979 A (DING et al) 12 December 1995 (12/12/95), see column 3, lines 58-63, example 1, column 5, lines 29-32.	1-3,6
X,P	US 5,583,105 A (KOVACS et al) 10 December 1996 (10/12/96), see column 2, line 49 - column 3, line 6, column 3, lines 26-32.	1,2,4,6,7
X,P	US 5,540,931 A (HEWITT et al) 30 July 1996 (30/07/96), see column 6, lines 37-43, column 7, lines 3-8.	1-4, 6-8, 11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/04627

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG

search terms: cyclosporin, ethanol, propylene glycol, polyoxyethylene sorbitan, TWEEN, dispersion, emulsion, nanoemulsion, nanoparticle, microparticle, oral, amorphous, noncrystalline